IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Jeffrey A. Smith, et al.	*Customer No.*	34444
Serial No.	10/517,328	*Art Unit:*	1623
Filing Date:	December 9, 2004	*Examiner:*	Ganapathy Krishnan

Title: RSK Inhibitors and Therapeutic Uses Thereof

AFFIDAVIT UNDER 37 CFR § 1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Dear Sir:

We, Jeffrey Smith and Deborah Lannigan declare and state as follows.

1. Jeffrey Smith is a Visiting Scientist at the University of Virginia and Deborah Lannigan is an Associate Professor at the University of Virginia and we are named co-inventors of the above-identified patent application.

2. We each have Ph.D.s. and our combined experience includes close to 50 years of research. We have each worked in the fields of cellular and molecular biology for most of that time and have worked in the specific research area encompassed by the present patent application for many years.

3. A listing of our education, publications, projects, awards, and work history are provided in our Curriculum Vitaes, both of which are in the attached Appendix.

5. We are familiar with the prosecution of the above-identified Application and have read and understand the Office Action issued April 19, 2007.

6. We participated in the recent telephonic interview which included attorney Rodney Sparks and Examiner Krishnan.

7. We understand that the Examiner has rejected all claims (21-28, 32, and 51-53) as either lacking enablement or as being obvious over various combinations of Matthes, Bjorbaek, Marks, Kuijpers and Pienta.

For the reasons discussed below and based on the data provided below, we believe that the specification enables the claims for multiple cancers using multiple inhibitors of RSK activity and that the claims are not obvious, because the combination of references cited by the Examiner does not render the claims obvious and further that one of the references (Matthes) and other art (Dai et al., discussed in the Response) teach away from the present invention as claimed.

8. ***The Invention***

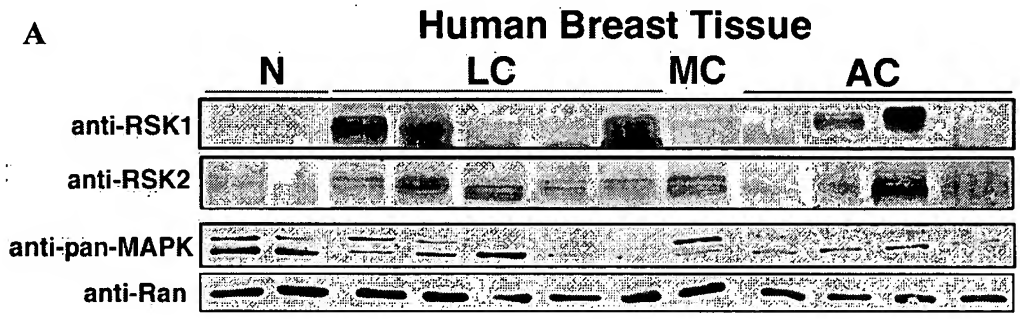
This invention, as stated in the application, was the first demonstration that the RSK family, through regulation of its downstream effectors, is involved in the control of cancer cell proliferation and is supported by several unexpected discoveries disclosed in the specification as filed and as discussed in the accompanying Response.

9. ***New Data and Recent Art Supporting the Invention as Claimed***

Four basic concepts are discussed below: 1) During our continued research of RSK inhibitors as anti-cancer agents we have now demonstrated that RSK is over expressed in many more clinically-isolated human tumor tissues relative to the levels detected in normal human tissues, and not just the three types of tumors disclosed in the specification; 2) We have also synthesized multiple SL0101 analogs; seven of which inhibit RSK activity by 50% at concentrations less than 5 μM; 3) Additionally, we have demonstrated that inhibition of RSK activity inhibits the growth of many more cancer cell lines derived from numerous tissues. These data are detailed in this declaration; 4) Since the filing of this application, others have further verified the observations in the application, providing further evidence that the specification adequately enables the invention as claimed. Several of these studies by others are described below.

10. ***Excessive Expression of RSK***

The present specification states that malignant transformation and progression in human cancers are frequently associated with over-abundance or excessive activity of proteins that are involved in normal cellular processes. We have demonstrated RSK levels are increased in more than 50% of clinically-obtained samples from human breast, prostate and ovarian cancer tissue compared to the RSK levels in non-cancerous tissues. These data are presented in Figures 1-3 provided below.



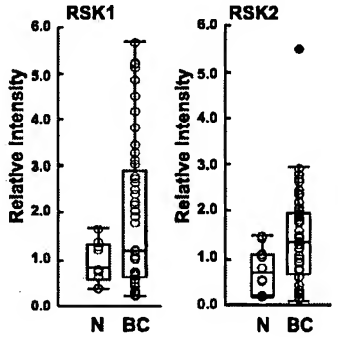
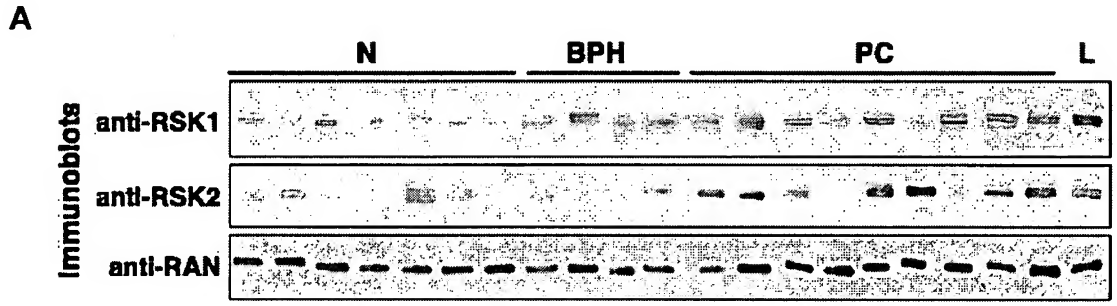


Figure 1. RSK is over expressed in human breast cancer. *(A)* N, normal breast (*n=12*) and BC, breast cancer (*BC*; consisting of ductal and lobular carcinoma) (n=48) tissue samples were ground under liquid nitrogen and lysed in SDS loading buffer. Samples were normalized to RAN and the normalized samples immunoblotted. A representative subset is shown. *(B)* Immunoblots were analyzed by densitometry, and the normalized intensities of RSK were plotted as box-and-whisker plots. *Bar in the box,* statistical median; *ends of the box,* upper and lower quartiles. *Solid circle in the RSK2 plot,* statistical outlier and not included in the SD. Box-and-whisker plots clearly illustrate the range of the data. Mean of RSK1 and RSK2 levels in the cancer tissue samples and mean in the normal tissue samples are statistically different. $P \leq 0.05$ (Student's *t* test, two-tailed distribution). ***(Smith, et. al., 2005)***



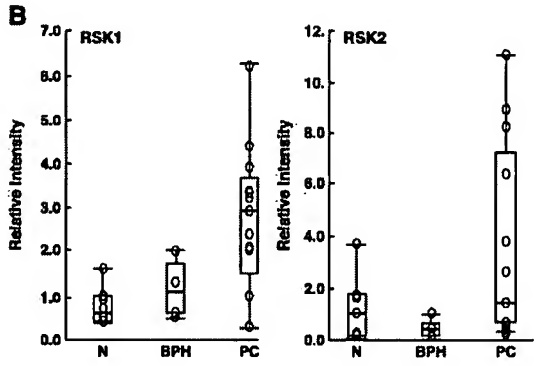


Figure 2. RSK levels are higher in human prostate cancer tissue. *(A) N,* normal tissue (n = 7); *BPH,* benign hyperplastic prostate tissue (n = 4); *PC,* prostate cancer tissue (n = 13); *L,* LNCaP cell line samples were ground under liquid nitrogen and lysed in SDS loading buffer. Samples were normalized to RAN and the normalized samples immunoblotted. A representative subset is shown. *(B),* Immunoblots were analyzed by densitometry and normalized intensities or RSK plotted as a box-and-whisker plot. *Bar in the box,* statistical median; *ends of the box* upper and lower quartiles. ***(Clark, et. al., 2005)***

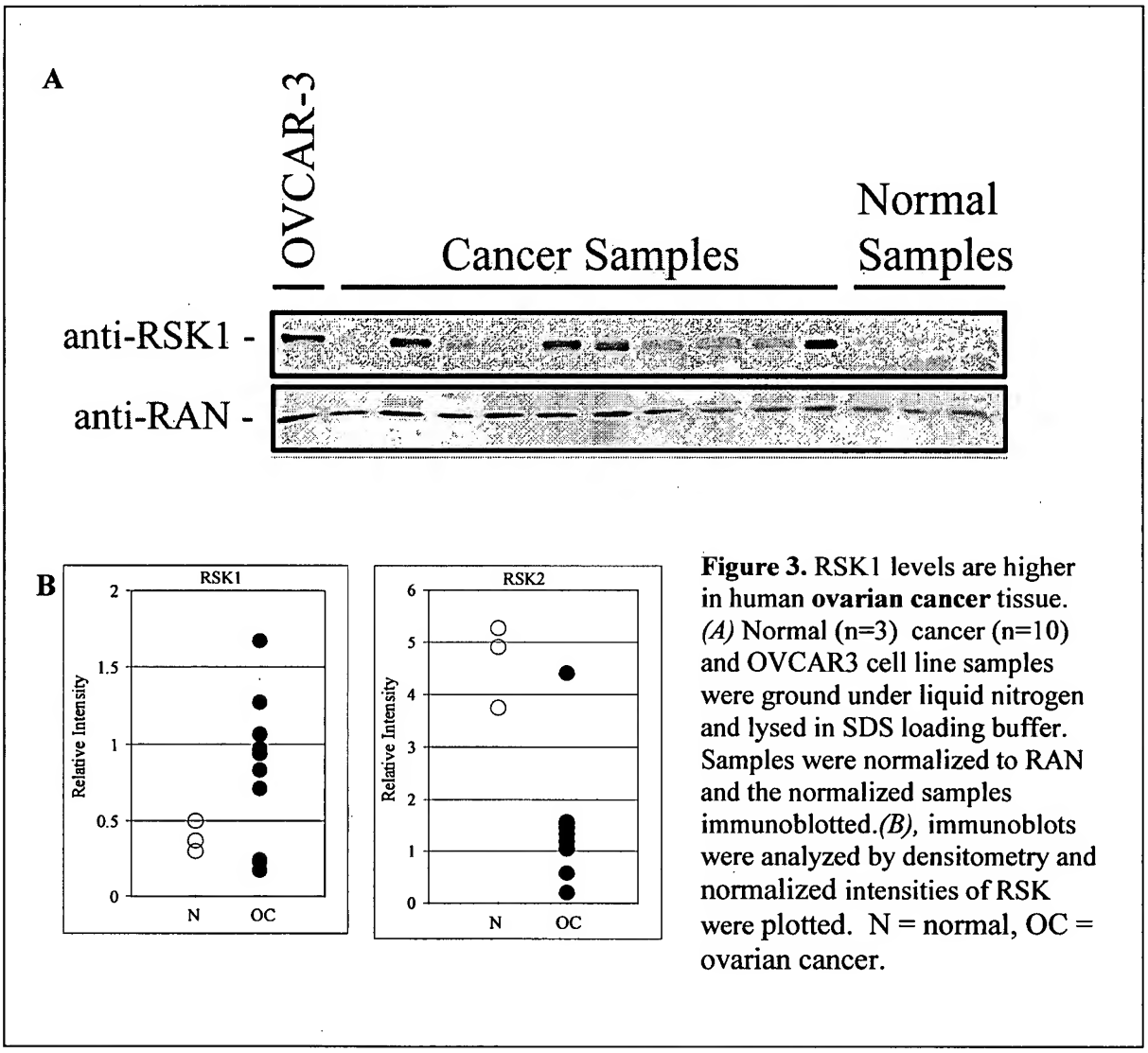
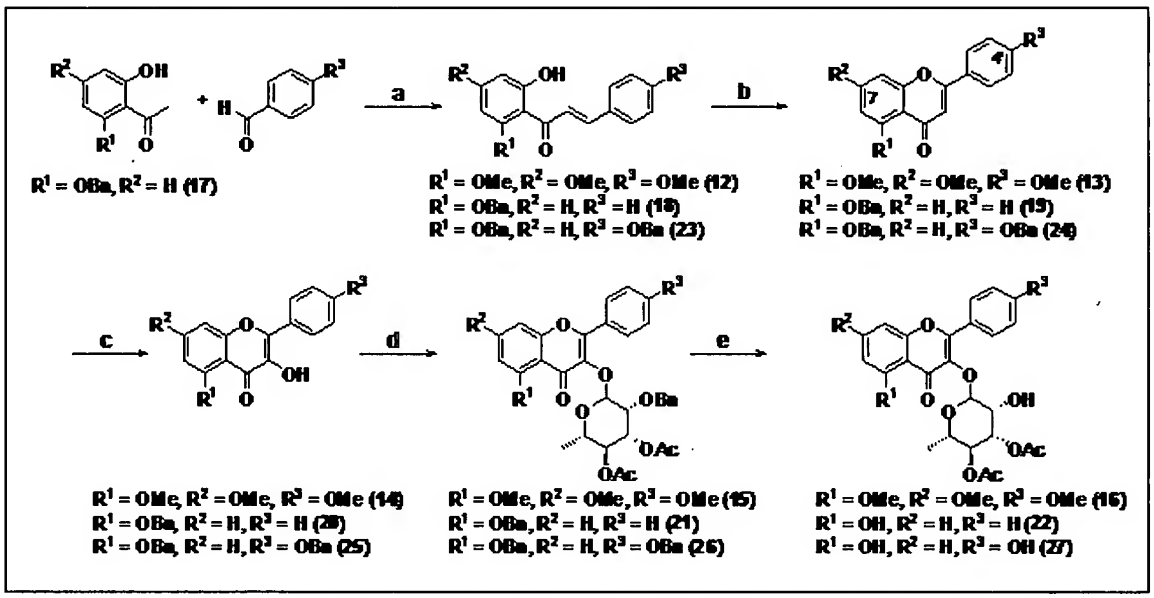


Figure 3. RSK1 levels are higher in human **ovarian cancer** tissue. *(A)* Normal (n=3) cancer (n=10) and OVCAR3 cell line samples were ground under liquid nitrogen and lysed in SDS loading buffer. Samples were normalized to RAN and the normalized samples immunoblotted.*(B),* immunoblots were analyzed by densitometry and normalized intensities of RSK were plotted. N = normal, OC = ovarian cancer.

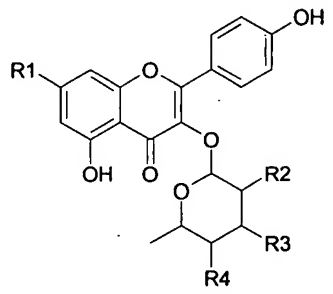
11. ***Inhibitory Potency of SL0101 Analogs and their Synthesis***

During the course of these studies, we identified **seven SL0101 analogs** that inhibit RSK activity by 50% at concentrations less than 5 μM. Thus, as indicated in the present specification, SL0101 analogs are potent RSK inhibitors. These analogs were synthesized using variations of the general synthetic scheme used for SL0101 (Scheme 1; see also Specification). The structures and inhibitory potencies of the analogs are depicted in Table 1.



Scheme 1. Conditions: (a) 40% w/v KOH, MeOH, reflux; (b) I_2, DMSO, 140°C; (c) i) DMDO (0.05-0.1 M in acetone), CH_2Cl_2, 4°C; ii) pTsOH, $CHCl_3$; (d) Ag_2, 4A mol. Sieves, CH_2Cl_2; (e) $Pd(OH)_2/C$, H_2, 1:1 THF-MeOH.

Table 1.



COMPOUND	R1	R2	R3	R4	IC50
SL0101	OH	OH	O-Acetyl	O-Acetyl	0.09 μM
Analog 1	OH	OH	O-Ethyl	O-Ethyl	0.13 μM
Analog 2	OH	OH	OH	O-Acetyl	0.14 μM
Analog 3	OH	OH	O-Butyryl	O-Butyryl	0.21 μM
Analog 4	OH	O-Acetyl	OH	O-Acetyl	0.26 μM
Analog 5	OH	O-Acetyl	O-Acetyl	O-Acetyl	0.58 μM
Analog 6	-	OH	O-Acetyl	O-Acetyl	1.56 μM
Analog 7	OH	OH	OH	OH	4.37 μM

Table 1. Inhibition potencies of SL0101 analogs. Kinase assays: Glutathione-S-transferase (GST)-fusion protein (1 μg) containing the sequence - RRRLASTNDKG (for serine/threonine kinase assays) was adsorbed in the wells of LumiNunc 96-well polystyrene plates (MaxiSorp surface treatment). The wells were blocked with sterile 3% tryptone in phosphate buffered saline. Kinase (5 nM) in 70 μL of kinase buffer (5 mM β-glycerophosphate pH 7.4, 25 mM HEPES, pH 7.4, 1.5 mM DTT, 30 mM $MgCl_2$, 0.15 M NaCl) was dispensed into each well. Compound at the indicated concentrations or vehicle was added and reactions were initiated by the addition of 30 μL of ATP to a final ATP concentration of 10 μM. Reactions were terminated after 30 min by addition of 75 μL of 500 mM EDTA, pH 7.5. All assays measured the initial velocity of reaction. After extensive washing of wells, anti-p-p140 antibody, a polyclonal phosphospecific antibody developed against the phosphopeptide, CGLApSTND, and HRP-conjugated anti-rabbit antibody (211-035-109, Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania) were used to detect serine phosphorylation of the substrate. HRP activity was measured using Western Lightning Chemiluminescence Reagent (NEL102, PerkinElmer Life Sciences) according to the manufacturer's protocol. Maximum and minimum activity is the relative luminescence detected in the presence of vehicle and 200 mM EDTA, respectively. His-tagged active RSK was expressed in Sf9 cells and purified using NiNTA resin (Qiagen, Valencia, California). Baculovirus was prepared using the Bac-to-Bac® baculovirus expression system (Invitrogen, Carlsbad, California). Maximum responses and the concentrations at half the inhibitory response (IC_{50}) were determined by performing a best-fit analysis of the data (GraphPad Prism). ***(Smith, et. al., 2007)***

12. ***Inhibition of Cancer Cell Proliferation***

We have demonstrated in our laboratory and in collaboration with the National Cancer Institute (NCI) that using routine assays the growth of numerous cancer cell lines from various tissues are sensitive to RSK inhibition. As seen in Table 2, the growth of thirty human cancer cells lines originating from 9 different tissues is inhibited by 50% at concentrations of a SL0101 analog (Analog 5 from Table 1) of less than 25 μM. Therefore, we have confirmed empirically that RSK inhibitors can be used to inhibit the growth of a variety of cancers.

Table 2.

	GI_{50} Average	Std Dev		GI_{50} Average	Std Dev
LEUKEMIA			**MELANOMA**		
CCRF-CEM	7.8 μM	3 μM	LOX IMVI	16 μM	3 μM
K-562	8.3 μM	3 μM	MALME-3	18 μM	0.7 μM
MOLT-4	24 μM	0.5 μM	SK-MEL-5	15 μM	3 μM
RPMI-8226	7.6 μM	2 μM			
			OVARIAN		
LUNG, non-small cell			IGROV1	21 μM	0.1 μM
HOP-62	22 μM	0.1 μM	OVCAR-4	21 μM	21 μM
HOP-92	9 μM	1 μM			
NCI-H23	23 μM	1 μM	**RENAL**		
NCI-H322M	21 μM	1 μM	768-0	23 μM	2 μM
NCI-H460	17 μM	2 μM	ACHN	22 μM	3 μM
NCI-H522	17 μM	2 μM	CAKI-1	23 μM	4 μM
			SN12C	21 μM	0.2 μM
COLON			TK-10	22 μM	0.07 μM
HCC-2998	21 μM	5 μM			
HCT-116	8.7 μM	3 μM	**PROSTATE**		
HCT-15	19 μM	3 μM	PC-3	12 μM	4 μM
KM12	19 μM	0.4 μM			
			BREAST		
CNS			NCI/ADR-RES	21 μM	0.07 μM
SF-295	24 μM	3 μM	MDA-MB-435	22 μM	2 μM
U251	21 μM	0.9 μM	T-47D	21 μM	6 μM

Table 2. Inhibition of Cancer Cell Proliferation by SL0101 analogs. For proliferation studies cells were seeded at 5000 cells per well in 96 well tissue culture plates in RPMI. After 24 h, the medium was replaced with medium containing vehicle or varying concentrations of compound. Cell viability was measured 48 h later. The concentration of compound required to inhibit growth by 50% (GI_{50}) is indicated.

13. **Corroboration of Applicants' Invention by Others** - Since our initial discovery that RSK regulates the growth of cancer cells, other groups have confirmed our observations. These studies include:

1) Tumor-associated angiogenesis

Hayashi, M., Fearns, C., Eliceiri, B., Yang, Y. and Lee, J. D. 2005. Big mitogen-activated protein kinase 1/extracellular signal-regulated kinase 5 signaling pathway is essential for tumor-associated angiogenesis. Cancer Res 65:7699-706. The authors demonstrate that activity of Big Mitogen-activated Protein Kinase 1/ ERK5 is required for tumor-induced angiogenesis. The vasculature of mice in which BMK1/ERK5 expression is eliminated through genetic alteration does not invade a xenografted tumor whereas the tumor induces neo-vascularization in wild type mice. The defect in tumor-induced endothelial cell proliferation was traced to reduced RSK activity in the absence of BMK1/ERK5. Thus, inhibition of RSK activity reduces tumor-induced angiogenesis.

2) Transformation of cells

Jackson, M. W., Patt, L. E., LaRusch, G. A., Donner, D. B., Stark, G. R. and Mayo, L. D. 2006. Hdm2 nuclear export, regulated by insulin-like growth factor-I/MAPK/p90Rsk signaling, mediates the transformation of human cells. J Biol Chem 281:16814-20. The authors determined that RSK activity resulted in the cytoplasmic accumulation and increased expression of the oncoprotein, Hdm2. Hdm2 is involved in the destruction of the tumor suppressor, p53. Thus, RSK activity results in decreased protein levels of p53, which reduces p53-mediated regulation of proliferation. Expression of inactive RSK inhibited the Hdm2-mediated degradation of p53. Remarkably, the authors determined that expressing constitutively active RSK in the context of oncogenic H-Ras promoted anchorage-independent growth in human fibroblasts. The authors conclude that elevated MAPK, and therefore RSK activity will "dramatically affect the p53-Hdm2 axis in favor of attenuating p53 activity, which contributes to the progression of tumor formation and may provide a novel point for therapeutic intervention."

Ribosomal S6 Kinase 2 is a key regulator in tumor promoter-induced cell transformation. 2007 Cancer Res 67:8104-12. Cho, Y-Y., Yao, K., Kim, H-G., Kang, B-S., Zheng, D., Bode, A.M., and Dong, Z. Over expression or constitutive activation of components within the MAPK pathway are observed in numerous tumors. The authors demonstrate that ectopic expression of RSK2 is sufficient to increase proliferation and induce anchorage-independent growth in the non-transformed mouse epidermal cell line, JB6 Cl41 cells. The number of foci induced by RSK expression far exceeded that induced by epidermal growth factor or phorbol ester treatment. Additionally, increasing RSK expression in NIH 3T3 cells enhanced the number of foci induced by Ras transformation by greater than 3-fold. Interfering with RSK2 expression using RSK2-specific siRNA eliminated the transformation induced by constitutively active Ras. The authors conclude that RSK2 is a key regulator of cell transformation induced by dysregulation of MAPK pathway components.

FGFR3 Activates RSK2 to Mediate Hematopoietic Transformation through Tyrosine Phosphorylation of RSK2 and Activation of the MEK/ERK Pathway. 2007 Cancer Cell 12: 201-14. Kang S, Dong S, Gu TL, Guo A, Cohen MS, Lonial S, Khoury HJ, Fabbro D,

Gilliland DG, Bergsagel PL, Taunton J, Polakiewicz RD, Chen J. Summary written by Dr. Angel Nebreda (***2007 Cancer Cell 12: 187-89)*** The tyrosine kinase receptor FGFR3 is thought to play a role in hematopoietic malignancies. A new study in this issue of Cancer Cell identifies the serine/threonine kinase RSK2 as a key substrate of FGFR3 in human (4;14)-positive multiple myeloma (MM) cells. Constitutively active FGFR3 directly phosphorylates RSK2 on Tyr529, which primes RSK2 for activation by the kinases ERK1 and ERK2 (ERK1/2). In turn, RSK2 activity plays an important role in the survival of FGFR3-expressing MM cells.

3) Osteosarcoma development

David, J. P., Mehic, D., Bakiri, L., Schilling, A. F., Mandic, V., Priemel, M., Idarraga, M. H., Reschke, M. O., Hoffmann, O., Amling, M., et al. 2005. Essential role of RSK2 in c-Fos-dependent osteosarcoma development. J Clin Invest 115:664-72. It has been demonstrated that transgenic mice overexpressing the transcription factor, c-fos, develop spontaneous osteosarcomas Crossing the, c-fos transgenic mice with RSK2 knockout mice substantially reduces osteosarcoma proliferation. Thus, the lack of RSK2 activity is thought to be responsible for decreased proliferation and increased apoptosis of transformed osteoblasts. The authors conclude "molecules inhibiting RSK2 activity could be used to treat human osteosarcomas."

4) Non-small cell lung cancer

Hurbin, A., Coll, J. L., Dubrez-Daloz, L., Mari, B., Auberger, P., Brambilla, C. and Favrot, M. C. 2005. Cooperation of amphiregulin and insulin-like growth factor-1 inhibits Bax- and Bad-mediated apoptosis via a protein kinase C-dependent pathway in non-small cell lung cancer cells. J Biol Chem 280:19757-67. Amphiregulin (AR) and insulin-like growth factor-1 (IGF1) secreted by non-small cell lung cancer (NSCLC) cells are known to promote NSCLC survival. The authors show that interfering with RSK expression using siRNA blocks the survival activity of AR/IGF1 combination treatment. Thus, treating the cells with RSK inhibitors would also interfere with AR/IGF1-induced tumor cell survival.

5) Tumor Cell Survival

Anjum, R., Roux, P. P., Ballif, B. A., Gygi, S. P. and Blenis, J. 2005. The tumor suppressor DAP kinase is a target of RSK-mediated survival signaling. Curr Biol 15:1762-7. Death-associated Protein Kinase (DAPK) is a tumor suppressor involved in killing unhealthy cells. The authors demonstrate that DAPK is phosphorylated and inactivated by RSK. Therefore, RSK activity promotes tumor cell survival by inhibiting the ability of DAPK to induce programmed cell death of the compromised cell. Interfering with RSK expression using siRNA significantly reduced DAPK phosphorylation. The implication of these studies is that use of RSK inhibitors would allow DAPK to remain active and suppress tumor formation.

6) Reducing Drug Resistance

Inhibition of the mitogen-activated protein kinase pathway results in the down-regulation of P-glycoprotein. 2007. Mol Cancer Ther 6:2092-102. Katayama, K., Yoshioka, S., Tsukahara, S., Mitsuhashi, J., and Sugimoto, Y. The multidrug transporter P-glycoprotein (P-gp) is one of several proteins that pump anticancer agents out of the cytoplasm reducing the intracellular concentration of the drugs. Cancer cells that express P-gp are often resistant to the cytotoxic effects of anticancer drugs. The authors demonstrate that interfering with RSK expression using RSK-specific short, interfering RNA (siRNA) decreased levels of P-gp on the surface of human colorectal and breast cancer cells. Pharmacological inhibition of ERK, the upstream activator of RSK, results in increased cellular uptake of rhodamine 123 as well as increased sensitivity of the cells to paclitaxel-induced apoptosis. These data demonstrate that

inhibiting RSK reduces the ability of the cells to remove the anticancer agents from the cytoplasm and therefore increases the sensitivity of the drug-resistant cells to chemotherapeutic agents. The authors state that the data provide new insight in to P-gp regulation as well as new strategies "for the reversal of P-gp-mediated anticancer drug resistance".

14. ***Literature Cited:***

Clark, D. E., Errington, T. M., Smith, J. A., Frierson, H. F., Jr., Weber, M. J. and Lannigan, D. A. 2005. The serine/threonine protein kinase, p90 ribosomal S6 kinase, is an important regulator of prostate cancer cell proliferation. Cancer Res **65:**3108-16.

Smith, J. A., Poteet-Smith, C. E., Xu, Y., Errington, T. M., Hecht, S. M. and Lannigan, D. A. 2005. Identification of the first inhibitor of p90 ribosomal S6 kinase (RSK) reveals an unexpected role for RSK in cancer cell proliferation. Cancer Res. **65:**1027-1034.

Smith, J. A., Maloney, D. J., Hecht, S. M. and Lannigan, D. A. 2007. Structural basis for the activity of the RSK-specific inhibitor, SL0101. Bioorg Med Chem **15:**5018-34.

Copies of all articles cited herein are being provided with this Declaration which is being submitted with a response to the Office Action dated April 19, 2007, a Supplementary IDS, as well as copies of our curriculum vitaes.

15. For the reasons described, we believe that the specification teaches how to make and use Rsk inhibitors as claimed, that it teaches how to test cancers for excessive Rsk activity prior to treatment, that it teaches that multiple cancers can be treated once found to have excessive Rsk activity, and that our new data and those of others indicate that no undue experimentation is required to do so.

We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code that such willful false statements may jeopardize the validity of the application of any patent issued thereon.

Respectfully submitted,

Date: 9-18-07

Jeffrey Smith

Date: 9-19-07

Deborah Lannigan

CURRICULUM VITAE
Jeffrey A. Smith, Ph.D.

I. Personal Data

Jeffrey Allan Smith, Ph.D.
815 Stillwater Lane
Earlysville, VA 22936
Phone: 434.964.1523
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II. Academic Appointments

August 2005 – present	Visiting Scientist, University of Virginia
January 2002 – August 2005	Research Assistant Professor, Department of Pathology University of Virginia
October 1999 - January 2002	Senior Scientist, Center for Cell Signaling University of Virginia

III. Other Employment Pertaining to Current Professional Appointments

August 2005 - present	Drug Discovery Supervisor, Luna Innovations, Incorporated Roanoke, VA
October 1986 –August 1990	Technical Assistant, Howard Hughes Medical Institute Vanderbilt University School of Medicine John H. Exton, M.D., Ph.D.

IV. Education

1986	B.S.	David Lipscomb College, Nashville, TN
1996	Ph.D.	Vanderbilt University Medical School Nashville, TN Mentor: Jackie D. Corbin, Ph.D.

V. Post-Graduate Education

1996 - 1999	Howard Hughes Medical Institute University of Virginia, Thomas W. Sturgill, M.D., Ph.D.

VI. Areas of Research Interest

Tumorigenesis and Progression; Septicemia; Angiogenesis; Inflammation; Bacterial Pathogenesis; Design of *In Vitro* and Cell-based Assays

VII. Teaching Activities

During my 10 years at the University of Virginia I have been responsible for supervising numerous undergraduate students, graduate students, technicians and post-doctoral scientists. As Drug Discovery Supervisor at Luna Innovations, I manage the daily research activities of two Ph.D.-level scientists.

VIII. Current Projects

Development of Anti-*Yersinia pestis* therapeutics
Discovery of Small-molecule Angiogenesis Inhibitors
Development of Kinase-specific Markers for Cell-based Assays

IX. Financial Resources (Grants and Contracts)

ACTIVE:

W81WXH-04-C-0049 01/19/06 – 02/22/08
Jeffrey A. Smith, PI
USA Med Research Acq Activity
"Isolation of Focal Adhesion Kinase Specific Modulators".

Jeffrey A. Smith, PI 06/07 – 11/07
NIH-NIGMS
"Development of Kinase-specific Molecular Markers for Cell-based Assays"

COMPLETED:

1 R43 AI066612-01 08/10/05-12/31/06
Jeffrey A. Smith, PI
NIH-NIAID
"Validation of a RSK-inhibitor as a Novel Therapeutic for Yersinia".

CaP CURE Smith (co-PI) 06/01/03-05/31/04
"Pre-clinical Evaluation of a Novel, Specific, Small-molecule Inhibitor of the Protein Kinase, RSK, for Prostate Cancer Treatment"

R21 PAR-01-045 Smith (co-PI) 05/01/02-04/31/04
NIH/NCI
"Identification of Inhibitors for the RSK2 Protein Kinase"

X. Patents Filed

p90Rsk inhibitors and therapeutic uses thereof. Inventors: J. A. Smith, D.A. Lannigan, C. E. Poteet-Smith, S. Hecht, D. Brautigan, & Y. Xu., filed June, 2003.

ERK7/8, Novel Therapeutic Targets for Cancer Chemotherapy. Inventors: D.A. Lannigan, J.A. Smith and L.M. Henrich., filed June, 2004.

Influence of Rhamnose Substituents on the Potency of SL0101, an inhibitor of the Ser/Thr Kinase, RSK. Inventors: J. A. Smith, D.A. Lannigan, D. Maloney, S. Hecht., filed May, 2006.

Trade Secrets and Invention Disclosures filed: 8

XI. Papers Published or in Press

1) Lynch, C.J., Taylor, S.J., **Smith, J.A.**, Exton, J.H. (1988) Formation of the high-affinity agonist state of the alpha 1-adrenergic receptor at cold temperature does not require a G-protein. *FEBS Lett* 229: 54-58.
2) Taylor, S.J., **Smith, J.A.**, Exton, J.H. (1990) Purification from bovine liver membranes of a guanine nucleotide-dependent activator of phosphoinositide-specific phospholipase C. Immunologic identification as a novel G-protein subunit. *J Biol Chem* 265: 17150-17156. **(Cited 13 times; Google Scholar Search)**
3) **Smith, J.A.**, Francis, S. H., Corbin, J.D. (1993) Autophosphorylation: a salient feature of protein kinases. *Mol. Cell Biochem.* 127/128: 51-70. **(Cited 26 times; Google Scholar Search)**
4) **Smith, J.A.**, Francis, S.H., Walsh, K.A., Kumar, S., Corbin, J.D. (1996) Autophosphorylation of type I β cGMP-dependent protein kinase increases basal catalytic activity and enhances allosteric activation by cGMP or cAMP. *J. Biol. Chem.* 271: 20756-20762. **(Cited 41 times; Google Scholar Search)**
5) Francis, S.H., **Smith, J.A.**, Colbran, J.L., Grimes, K., Walsh, K.A., Kumar, S., Corbin, J.D. (1996) Arginine-75 in the pseudosubstrate sequence of type I β cGMP-dependent protein kinase provides for potent autoinhibition, but the primary autophosphorylation at Ser-63 is well outside this sequence. *J. Biol. Chem.* 271: 20748-20755. **(Cited 23 times; Google Scholar Search)**
6) Joel, P., **Smith, J.**, Sturgill, T.W., Fisher, T.L., Blenis, J., and Lannigan, D.A. (1998) pp90rsk1 regulates estrogen receptor-mediated transcription through phosphorylation of Ser-167. *Mol. Cell Biol.* 18: 1978-1984. **(Cited 145 times; Google Scholar Search)**
7) **Smith, J.A.**, Poteet-Smith, C.E., Malarkey, K., and Sturgill, T.W. (1999) Identification of an extracellular signal-regulated kinase (Erk) docking site in ribosomal S6 kinase, a sequence critical for activation by Erk *in vivo*. *J. Biol. Chem.* 274: 2893-2898. **(Cited 124 times; Google Scholar Search)**
8) Poteet-Smith, C.E.*, **Smith, J. A.***, Lannigan, D.A., Freed, T.A. and Sturgill, T.W. (1999) Generation of constitutively active p90 ribosomal S6 kinase in vivo; implications for the MAP kinase-activated protein kinase family. *J. Biol. Chem.* 274: 22135-22138. **(Cited 30 times; Google Scholar Search)**
*** = equal contributors**
9) **Smith, J.A.**, Reed, R.B., Francis, S.H., Grimes, K., Corbin J.D. (2000) Distinguishing the roles of the two different cGMP-binding sites for modulating phosphorylation of exogenous substrate (heterophosphorylation) and autophosphorylation of cGMP-dependent protein kinase. *J. Biol. Chem.* 275 (1):154-158. **(Cited 8 times; Google Scholar Search)**

10) Steiner, T.S., Nataro, J.P., Poteet-Smith, C.E., **Smith, J.A.**, and Guerrant, R.L. (2000) Enteroaggregative *Escherichia coli* Expresses a Novel Flagellin That Causes IL-8 Release From Intestinal Epithelial Cells. *J Clin. Invest.* **105**: 1769-1777. **(Cited 81 times; Google Scholar Search)**

11) **Smith, J.A.**, Poteet-Smith, C.E., Lannigan, D.A., Freed, T.A., Zoltoski, A.J., and Sturgill, T.W. (2000) Creation of A Stress-activated p90 Ribosomal S6 Kinase: The Carboxyl-Terminal Tail of the MAPKAPKs Dictates The Signal Transduction Pathway In Which They Function. *J. Biol. Chem* .275 (41): 31588-31593. **(Cited 14 times; Google Scholar Search)**

12) Clark, D.E., Poteet-Smith, C.E., **Smith, J.A.**, and Lannigan, D.A. (2001) Rsk2 Allosterically Activates Estrogen Receptor α by Docking to the Hormone Binding Domain. *EMBO J.* 20(13): 3484-3494. **(Cited 14 times; Google Scholar Search)**

13) Henrich, L.M., **Smith, J. A.**, Kitt, D., Errington, T., Nguyen, B., Traish, A. M., and Lannigan, D. A. (2003) Extracellular signal-regulated kinase7, a regulator of hormone-dependent estrogen receptor. *Mol. Cell. Biol.* 23:5979-5988. **(Cited 9 times; Google Scholar Search)**

14) **Smith, J.A.**, Poteet-Smith, C.E., Xu, Y., Errington, T.M., Hecht, S.M. and Lannigan, D.A. (2005) Identification of the First Specific Inhibitor of p90 Ribosomal S6 Kinase (RSK) Reveals an Unexpected Role for RSK in Cancer Cell Proliferation. *Cancer Res.* 65:1027-1034. **(Cited 6 times; Google Scholar Search)**

15) Clark, D.E., Errington, T., **Smith, J.A.**, Frierson, H., Weber, M.J. and Lannigan, D.A. (2005) The Serine/Threonine Protein Kinase p90 Ribosomal S6 Kinase, is an important Regulator of Prostate Cancer Cell Growth. *Cancer Res.* 65, 3108-3116. **(Cited 4 times; Google Scholar Search)**

16) Xu, Y., **Smith, J.A.**, Lannigan, D.A. and Hecht, S.M. (2006) Three acetylated flavonol glycosides from *Forsteronia refracta* that specifically inhibit p90RSK. *Bioorg. Med. Chem.* 14(11):3974-7.

17) **Smith, J.A.**, Maloney, D.J., Clark, D.E., Xu, Y., Hecht, S.M. and Lannigan, D.A. (2006) Influence of rhamnose substituents on the potency of SL0101, an inhibitor of the Ser/Thr kinase, RSK. *Bioorg. Med. Chem.* 14(17):6034-42.

18) Nguyen, T.L., Gussio, R., **Smith, J.A.**, Lannigan, D.L., Hecht, S.M., Scudiero, D.A., Shoemaker, R.H., Zaharevitz, D.W., (2006) Homology model of RSK2 N-terminal kinase domain, structure-based identification of novel RSK2 inhibitors, and preliminary common pharmacophore. *Bioorg. Med. Chem.* 14(17):6097-105.

19) **Smith, J.A.**, Maloney, Hecht, S.M. and Lannigan, D.A. (2007) Structural basis for the activity of the RSK-specific inhibitor, SL0101. *Bioorg. Med. Chem.* 15:5018-34.

XII. Past Professional Experience

Drug Discovery Supervisor (8/2005 – Present) Luna Innovations Incorporated
Supervisor: Thomas A. Wavering; Vice President, Technology Development Division
Primary Duties: Program Management; Research and Development Logistics; Pre-clinical Research and Development
Planned and supervised the research activities of Drug Discovery team (3 Ph.D scientists); Managed the budgetary aspects of the programs; Developed and implemented research programs; Ensured compliance with Export Administration Regulations (EAR) and International Traffic in Arms Regulations (ITAR); Acquired internal and external funding to maintain research programs; Developed relationships with external collaborators; Coordinated and integrated collaborator research with that of the Drug Discovery team; Generated and protected novel intellectual property.

Assistant Professor of Research (1/2002 – 8/2005) University of Virginia
Supervisor: Deborah A. Lannigan, Ph.D.; Assistant Professor of Microbiology
Primary Duties: Drug Discovery
Performed high throughput screens (HTS) to identify novel chemical entities for therapeutics; Designed and validated novel HTS assays, Acquired internal and external funding to maintain the research program; Characterized the small molecule inhibitors in *in vitro* and cell-based assays; Published primary data articles; Collaborated with University of Virginia faculty for drug discovery-based research; Generated and protected novel intellectual property.

Senior Scientist (10/1999 – 1/2002) University of Virginia
Supervisor: Deborah A. Lannigan, Ph.D.; Assistant Professor of Microbiology
Primary Duties: Establish and automate the drug discovery program
Designed and validated novel HTS assays; Managed inventory of natural products extracts; Designed liquid handling and scheduling programs for automation of the assays; Achieved annual milestones to maintain funding.

HHMI Research Associate (11/1996 – 10/1999) University of Virginia
Supervisor: Thomas W. Sturgill, MD., Ph.D., Professor of Pharmacology
Primary duties: Post-Doctoral studies.
My post-doctoral studies were part of the early work that led to the realization that *in vivo* substrate specificity involves docking sites located distally to the substrate's phospho-acceptor sites and that signal transduction cascades are comprised of elaborate signaling complexes. I also designed a constitutively active RSK by mutating a single residue in what I identified as an auto-inhibitory domain. This reagent has been used by researchers across the United States and Europe as well as in Asia, the Middle East and South America to elucidate the physiological functions of RSK. These post-doctoral studies have been cited over 300 times.

CURRICULUM VITAE

DEBORAH A. LANNIGAN

PERSONAL INFORMATION

Office Address

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Center for Cell Signaling
P. O. Box 800577
Charlottesville, VA 22908-0577
Phone: (434) 924-1144
FAX: (434) 924-1236
email: dal5f@virginia.edu

Citizenship — Canadian, Permanent U.S. Resident
SSN 092724930

EDUCATION

Predoctoral Training

1980	B.Sc, Biochemistry, Summa Cum Laude University of Guelph, Ontario
1982	M.Sc., Biochemistry University of Toronto, Ontario
1987	Ph.D., Biophysics University of Rochester, New York (advisor: Dr. Philip Knauf)

Postdoctoral Training

1986-1991	Molecular Endocrinology University of Rochester, New York (advisor: Dr. Angelo Notides)

PROFESSIONAL EMPLOYMENT

1986-1991	**University of Rochester,** Research Associate, Environmental Health Sciences Center, Rochester, NY
1991-1995	**University of Vermont**, Assistant Professor, Department of Zoology, Burlington, VT
1995	**Receptor Technologies, Inc.**, Principal Scientist, Winooski, VT
1995-1996	**University of Vermont**, Research Assistant Professor of Pharmacology, Burlington, VT
1996-2002	**University of Virginia**, Research Assistant Professor of Pharmacology, Charlottesville, VA
1996-present	**University of Virginia**, Member, Center for Cell Signaling, Charlottesville, VA
2002-2007	**University of Virginia**, Assistant Professor of Microbiology, Charlottesville, VA
2007-present	**University of Virginia**, Associate Professor of Microbiology, Charlottesville, VA

AWARDS AND HONORS

1980	National Science and Engineering Research Council of Canada Studentship – a nationally competitive award given up in lieu of Medical Research Council Studentship
1980-1981	Medical Research Council of Canada Studentship – a nationally competitive award
1983-84	Program in Medicine and Biology Fellowship, University of Rochester, NY – A competitive predoctoral award
1990-1991	NIH Endocrinology Training Fellowship

EDUCATIONAL CONTRIBUTIONS

Course Teaching

University of Virginia

2004-2007	Medical Microbiology (4 lectures "Introductory Bacteriology")
2006, 2007	Summer Methods (1 lecture on "Drug Discovery")
2003-2007	Graduate Rotation Supervisor: Danielle Shingle, Matthew Crawford, Perry Kennedy, Stacy Berry, Angela Groehler, Derek Dube, Rebecca Davis, Sergio Sanchez, Karin Eisinger, Jenny Dressler, Valerie Siclari
2003-2006	MICR 815 Molecular Basis of Cancer (1 lecture "Drug Design")
2001-2004, 2007	Undergraduate Research Supervisor: Thuan Nguyen (College Science Scholar), Brian Marsh, Daniel Geary, Mary Jones, Chris Schabowsky, Bryce Galen
2005	"Cells to Society – An introduction to Medical School" (Small Group Leader)
2004	Mini-Med School Laboratory Tour
2002-2004	Summer Research Internship Program (1 lecture "Bioscreening and Biosensors")
2002-2003	Medical Microbiology, Laboratory Supervisor in "Bacteriology Section".

University of Vermont

1991	ZOO 371 Colloquium "How do steroid receptors regulate gene expression?" A literature-based seminar course. 1 credit. 4 graduate students.
1992-1995	BIO 101 "Genetics" An introductory genetics course which covered topics ranging from Mendelian genetics to molecular biology. Co-taught. 3 credits. Approx. 100 students per term.
1992	BIO 96 Colloquium "How do you get sex?" An introductory course for undergraduates on the molecular aspects of sexual development. 1 credit. 12 students.
1992-1994	BIO 267 "Molecular Endocrinology". A laboratory and lecture course which introduced students to the techniques of molecular and cellular biology and how these techniques are used to address questions in the field of endocrinology. This course was new, and I had to design and test all the experiments and write a lab manual that could be followed by undergraduate students. 4 credits. Approx. 12 students per term.
1992-1993	ZOO 197, Z00 198 Undergraduate Research Supervisor. 3 credits. 1 student.
1994	ZOO 96 "Women in the Life Sciences" An introductory course which overviewed contributions of women to the life sciences and discussed current issues of having a career in the life sciences.1 credit. 13 students.
1993-1994	ZOO 193, ZOO 194 Honors Undergraduate Research Supervisor . 3 credits. 2 students.
1995	ZOO 194 Undergraduate Research Supervisor. 1 credit. 1 student.
1995	ZOO 381 Colloquium "Critique of the current literature on small GTP-binding proteins". A literature-based seminar course. 3 credits. 2 students.

PROFESSIONAL SERVICES

Research Review Committees

2007	NCI Training Review Committee (Ad Hoc)
2007	Susan G. Komen Breast Cancer Foundation Research Grant Awards
2001,2006, 2007	Department of Defense Breast Cancer Research Program
2003-2007	Cancer Research United Kingdom
2002	Biochemical Endocrinology Study Section, NCI (Ad Hoc)
1999-2001	Susan G. Komen Breast Cancer Foundation Pre-Doctoral Awards
1999	Department of Defense Prostate Center Initiation Awards
1992-1996	American Heart Association, Research Committee, VT Affiliate

Meeting Planning Committees

2007	Department of Defense Prostate Cancer Innovative Minds in Prostate Cancer Today (IMPaCT)

Membership in National and International Academic Professional Organizations

1999-present	Biomolecular Screening
1993-present	Endocrine Society

Review manuscripts for: *Biochemistry, Current Medicinal Chemistry-Immunology, Endocrine, Endocrine & Metabolic Agents, European Molecular Biology Organization Journal, Federation of European Biochemical Societies Letters, Endocrinology, Journal of Biological Chemistry, Molecular Endocrinology, Molecular and Cellular Biology, Molecular Pharmacology, Sensors & Actuators:B. Chemical, Steroids*

ACADEMIC ADMINISTRATIVE ASSIGNMENTS AND RESPONSIBILITIES

Committees

University of Virginia

Faculty Advisory Committee for the Patent Foundation - Member
Thesis Committee Member - Natasha Schuh, Microbiology
- Huy Ta, Microbiology

University of Vermont

Thesis/Studies Committee Member for 3 students

Intramural Zoology Department

Ecology Faculty Search - Committee Member
Signal Transduction Faculty Searches - Committee Member
Zoology Seminar Series 92-95 - Chair
Undergraduate Retention Committee 93, 94 - Chair
Advisory Council for Zoology Department 94, 95 - Committee Member
Academic Advising 92-95 - 40 undergraduate students/year

Intramural University

Botany Faculty Search - Interviewed Candidates
Geology Faculty Search - Committee Member
Dean's Fund Committee 93 -95 - Committee Member
Women's Studies Committee Summer Undergraduate Internship - Chair [Established 2 fellowships]
Yield Day 92-95 - Zoology Faculty Representative
Graduate College- Committee Member of both the Executive Board and Standards Committee
Cell and Molecular Biology Graduate Program - Member of both Recruitment and Curriculum Committees

RESEARCH GRANTS

Active

a. N/A — 07/01/07-06/30/10 — 3.00 calendar
Susan G. Komen Breast Cancer Foundation — Direct Costs $345,718 – year 1
P.I. Macara; co-P.I. Lannigan
"Human Mammary Organoid Cultures-Genetic Manipulation and Live Imaging"
The goals of this grant are to test whether neighbor effects exist in human tissues using a novel model system that mimics human breast biology.

b. ARM-15-1040/612 — 01/15/06-01/15/08 — 0.6 calendar
USAMRMC- SBIR II — Direct Costs $49,555 – year 2
P.I. Lannigan
"FAK-specific modulators as angiogenic agents"
The goals of this grant are to identify and perform pre-clinical evaluation of FAK inhibitors.

c. N000140610888 — 06/16/06-06/15/09 — 0.60 calendar
ONR — Direct Costs $63,274 – year 2
P.I. Lannigan
"Modular yeast-based detection system for xenobiotic chemicals"
The goals of this grant are to improve the sensitivity and selectivity of a prototype TNT biosensor.

d. R01 CA116566 — 02/01/07-01/31/10 — 0.6 calendar
NIH — Direct Costs $125,000 –year 1
P.I. Hecht; co-P.I. Lannigan
"Inhibitors of p90Rsk"
The goals of this grant are to synthesize and characterize in *in vitro* and in cell-based assays SL0101 derivatives and to support the continued screening efforts for RSK inhibitors.

e. R01 GM50526 — 05/01/07-04/30/11 — 1.20 calendar
NIH — Direct Costs $312,000 – year 1
P.I. Macara; co-P.I. Lannigan
"The RAN GTPase"
The goals of this grant are to study the regulation and function of RAN and its binding partners in nuclear import/export, and in mitosis.

f. SBIR I — 08/01/07-02/28/08 — 0.6 calendar
NIH — Direct Costs $20,000
P.I. Lannigan
"Development of kinase-specific molecular markers for cell-based assays"
The goals of this grant are to develop novel assays for use in drug development.

g. N/A — 05/01/07-04/31/08 — N/A
Swortzel — Direct Costs $50,000
P.I. Lannigan
"Novel diagnostic assay for RSK function in ovarian cancer"
The goals of this grant are to identify molecular markers of RSK function in ovarian cancer.

h. N/A 03/01/07-02/28/08 N/A
Cancer Center Pilot Direct Costs $34,975
P.I. Lannigan
"Pre-clinical evaluation of 3Ac-SL0101 for ovarian cancer"
The goals of this grant are to establish the efficacy of 3Ac-SL0101 to inhibit ovarian tumor growth and angiogenesis in a xenograft model.

i. NCI-R*A*N*D Ongoing Collaboration
NIH
P.I. Lannigan
"The protein kinase RSK family-novel drug targets for chemotherapy"
This grant is a work request to have NCI researchers perform experiments in support of the identification of novel RSK-specific inhibitors and for further evaluation of SL0101.

Pending

a. Submission Date June 2007
NIH
P.I. Lannigan; co-P.I. Macara
"RSK2 and Stress"
The goals of this grant are to determine the molecular mechanism by which RSK2 controls survival in response to oxidative stress.

Previous

a. **Instructional Incentive Grant**
1993 Co-PI Direct Costs - $5,300
b. **UVM Committee on Research and Scholarship**
1992 Direct Costs - $4,917;1994 Direct Costs - $4,934
c **UVM Dean's Fund**
1992 Direct Costs - $2,000;1994 Direct Costs - $2,000.
d. **VISIT Undergraduate Research Fellowship** (Andrá Stevenson/Sponsor: Lannigan)
1993 (Summer) Direct Costs -$5,000.
e. **Women's Studies Natural Science Undergraduate Research Fellowship** (S. Bush/Sponsor: Lannigan)
1993 (Summer) Direct Costs -$2,500.
f. **Lake Champlain Cancer Research Organization.**
1992 Direct Costs - $10,000; 1995 Direct Costs - $10,000.
g. **American Cancer Society - Vermont Chapter.**
1992 Direct Costs - $10,000; 1994 Direct Costs - $5,000.
h. **NIH Senior Scientist** (Dr. Peteranne Joel/Sponsor: Lannigan)
1992 - 1993 Direct Costs - $17,650.
i. **Pfizer Undergraduate Research Fellowship** (M. Wells/Sponsor: Lannigan)
1993 (Summer) Direct Costs - $5,000.
j. **NIH R29 CA55887**
1992-1998 Direct Costs - $350,000
k. **AHA Postdoctoral Fellowship** (Dr. Robin Abramson/Sponsor: Lannigan)
1992 Direct costs - $18,000; 1993 & 1994 Direct Costs - $22,000 per year
l. **SIMS Obesity NIDDKD 5P30 DK46188**
1993 Direct Costs - $16,823.

m. **VT EPSCoR Receptor - Mediated Signalling and Biotechnology Cluster.**
1994 Direct Costs - $37,575.
1994 Undergraduate Research Fellowship (Carrie Smith/Sponsor: D. Lannigan) - $2,250.
1995 Direct Costs -$38,034.

n. **Undergraduate Research Fellowship** (Carrie Smith/Sponsor : D. Lannigan)
1995 – Direct Costs $3,000.

o. **Army Research Office- 36821-LS**
1996 – 2000 Direct Costs - $158,946

p. **American Cancer Society – Virginia Chapter**
1998 – Direct Costs $20,000

q. **Research and Development**
1998 – Direct Costs $10,000

r. **Assert**
1998 – 2001 Direct Costs - $62,187

s. **Luna Innovations**
1999 – Direct Costs $31,556

t. **Department of Defense – Fluorometer Equipment Grant**
2000- Direct Costs $114,330

u. **American Cancer Society RSG TBE-103393**
1999 to 2002- Direct Costs $300,000

v **ARGONEX**
1999 to 2002 – Direct Costs $412,922

w. **Rohm & Haas**
2000 to 2003 – Direct Costs $177,233

x. **LUNA Innovations**
2000 to 2002 – Direct Costs $157,195

y. **NIH -1R21 CA95335-01**
2002-2004 – Direct Costs $200,000

z. **Department of Defense SBIR I**
"Yeast-Based Biosensors for Organophosphate Detection"
2003-2003 – Direct Costs $20,270

aa. **Kincaid Research Grant**
2002-2003 – Direct Costs $19,365

bb. **Cancer Center Grant**
2002-2003 – Direct Costs $24,775

cc. **Cancer Center Grant**
2002-2003 – Direct Costs $75,000

dd. **Department of Defense –Breast Cancer**
"The Protein Kinase, RSK2, A Novel Drug Target for Breast Cancer'
2003-2006 – Direct Costs $287,771

ee. **Army Research Office**
"Explosive Residue Detection Using Polypeptide-Based Biosensors"
2000-2004 – Direct Costs $189,187

ff. **Department of Defense – SBIR II**
"Yeast-Based Biosensors for Organophosphate Detection"
2003-2005 – Direct Costs $152,026

gg. **CaPCURE**
2003- Direct Costs $50,000

hh. **Department of Defense – Prostate Cancer**
"The Protein Kinase RSK Family – Roles in Prostate Cancer"

2004-2007 – Direct Costs $371,370

ii. **Mellon Prostate Institute** 12/01/03 to 11/30/04
2003-2004 – Direct Costs $50,000

jj. **Department of Defense – SBIR I**
"FAK-specific modulators as angiogenic agents"
2004 – Direct Costs $15,091

kk. **NIAD-SBIR I**
"Validation of a RSK-inhibitor as a novel therapeutic for Yersinia"
2005-2006 – Direct Costs $93,596

RESEARCH SUPERVISORY ASSIGNMENTS

Personnel Currently Supervised

Jeffrey Smith, Ph.D.- Research Assistant Professor
David Clark – Laboratory and Research Specialist II
Lejla Pasic – Laboratory and Research Specialist I
Karin Eisinger – Graduate Student
Angela Groehler – Graduate Student
Brian Marsh – Univ. Virginia Undergraduate
Thuan Nguyen – Univ. Virginia Undergraduate (College Science Scholar)

Graduate Students

1993-1996	Sara Folta, M.S. Cell & Molecular Biology, Univ. Vermont
1994-1999	Nia Tatsis, Ph.D. Cell & Molecular Biology, Univ. Vermont
1999-2004	Lorin Henrich, Microbiology, Univ. Virginia
2004-present	Karin Eisinger, Microbiology, Univ. Virginia
2005-present	Angela Groehler, Molecular Medicine, Univ. Virginia

Postdoctoral Research Fellows

1993-1995	Robin Abramson, Ph.D.
1994-1998	Peteranne Joel, Ph.D.
2000-2002	Celeste Poteet-Smith, Ph.D.
1999-2001	Jeffrey Smith, Ph.D.
2001-2005	Siddhartha De, Ph.D.
2003-present	Josefa Andrade, Ph.D.
2006-present	Mark Mayhew, Ph.D.

Research Assistant Professor

2002-present	Jeffrey Smith, Ph.D.

INTELLECTUAL PROPERTY

Signal-generating oligonucleotide-based biosensors. Inventors: **D. A. Lannigan** and I. G. Macara. Patent awarded 09/529,870.

p90Rsk inhibitors as therapeutics and investigative tools. Inventors: **D.A. Lannigan**, D. Brautigan, S. Hecht, Y. Xu and J.A. Smith. PCT filed June 12, 2003.

Synthesis of 3Ac-SL0101. Inventors: **D.A. Lannigan**, J.A. Smith, D.J. Maloney and S.M. Hecht. Invention disclosure filed May, 2006.

Kinase-specific Molecular Markers for use in Cell-based Kinase Assays. Inventors:D.A. Lannigan, J.A. Smith and L.C. Cantley. Invention disclosure filed August, 2006.

RESEARCH PUBLICATIONS

Deber, C.M., Drobnies, A.E., Hughes, D.W. and **Lannigan, D.A.** (1982) Calcium complexation and transport by synthetic cyclic octapeptides. In: *Peptides:Synthesis, Structure, Function* (Rich,D.H. and Gross, E., eds.), Pierce Chemical Co., Rockford, IL, pp. 331-334.

Deber, C.M. and **Lannigan, D.A.** (1983) Uphill calcium transport in pressman cells by synthetic ionophorous peptide. In: *Physical Chemistry of Transmembrane Ion Motions* (Spach, G.,ed.), Elsevier Science Pub., Amsterdam, pp. 209-215.

Lannigan, D.A. and Knauf, P.A. (1985) Decreased intracellular Na^+ concentration is an early event in murine erythroleukemic cell differentiation. *J. Biol. Chem.* **260**: 7322-7324. (# citations -3) IF=5.854 (Rank 38 of 261 in the category Biochemistry and Molecular Biology).

Lannigan, D.A., Knauf, P.A. and Macara, I.G. (1986) Relationship of the decreases in proteinsynthesis and intracellular Na^+ during Friend murine erythroleukemic cell differentiation. *J.Biol. Chem.* **261**: 14430-14436. IF=5.854 (Rank 38 of 261 in the category Biochemistry and Molecular Biology).

Lannigan, D.A., Bennington, J.B., Cragoe, Jr., E.J. and Knauf, P.A. (1988) Phenamil, an amiloride analogue, inhibits differentiation of Friend murine erythroleukemic cells. *Am. J. Physiol.* **254**: 14430-14436. IF=3.942 (Rank 14 of 75 in Physiology).

Lannigan, D.A. and Notides, A.C. (1989) Estrogen receptor selectively binds to the "coding strand" of an estrogen responsive element. *Proc. Natl. Acad. Sci. USA* **86**: 863-867. (# citations -22) IF=10.231 (Rank 3 of 48 in Multidisciplinary Sciences).

Lannigan, D.A. and Notides, A.C. (1990) Estrogen regulation of transcription. In: *Progress in Clinical and Biological Research* (Stevens,J.L.,ed.), Alan R. Liss pp.187-197. (# citations -2)

Lannigan, D.A. and Notides, A.C. (1990) A novel mechanism for eukaryotic gene expression: The involvement of DNA tertiary structure in estrogen receptor recognition of its target nucleotide sequence. *Biochem. Pharm.* **40**: 2579-2585. (# citations -2) IF=3.617 (Rank 38 of 193 in Pharmacology and Pharmacy).

Lannigan, D.A., Tomashek, J.J., Obourn, J.D. and Notides, A.C. (1993) Analysis of estrogen receptor

interaction with tertiary structured estrogen responsive elements. *Biochem. Pharm.* **45**: 1921-1928. (# citations -3) IF=3.617 (Rank 38 of 193 in Pharmacology and Pharmacy).

Lannigan, D.A., Koszewski, N.J. and Notides, A.C. (1993) Estrogen responsive elements contain non-B DNA. *Mol. Cell. Endo.* **94**: 47-54. (# citations -4) IF=2.786 (Rank 35 of 89 in Endocrinology and Metabolism).

Joel, P.B., Traish, A.M. and **Lannigan, D.A.** (1995) Estradiol and phorbol ester cause phosphorylation of Serine 118 in the human estrogen receptor. *Mol. Endo.* **9**: 1041-1052. (# citations -56) IF=5.807 (Rank 9 of 89 in Endocrinology and Metabolism).

Bush, S.M., Folta, S. and **Lannigan, D.A.** (1995) Use of the yeast one-hybrid system to screen for mutations in the ligand-binding domain of the estrogen receptor. *Steroids* **61**: 102-109. (# citations-8) IF=2.416 (Rank 42 of 89 in the category Endocrinology and Metabolism).

Messier, T., Dorman, C., **Lannigan, D.A.** and Brann, M.R. (1996) Cell-based assays for G-protein coupled/ tyrosine kinase coupled receptors *J. Biomol. Screening.* **1**: 43-45. IF=2.763 (Rank 14 of 53 in Biochemical Research Methods). I help develop the cell-based assays.

Joel, P. B., Smith, J., Sturgill, T.W., Fisher, T. L., Blenis, J, and **Lannigan, D.A.** (1998) pp90^{rsk1} Regulates of estrogen receptor-mediated transcription through phosphorylation of Ser-167. *Mol. Cell. Biol.* **18**: 1978-1984. (# citations -150) IF=7.093 (Rank 19 of 153 in Cell Biology Category, Rank 27 of 263 in Biochemistry and Molecular Biology Category).

Joel, P. B., Traish, A. M., and **Lannigan, D.A.** (1998) Estradiol-induced phosphorylation of serine 118 in the estrogen receptor is independent of p42/p44 mitogen-activated protein kinase. *J. Biol. Chem.* **273**: 13317-13323. (# citations -79) IF=5.854 (Rank 38 of 261 in the category Biochemistry and Molecular Biology)

Tatsis, N., **Lannigan, D.A.** and Macara, I.G. (1998) The function of the p190 Rho GTPase-activating protein is controlled by its N-terminal GTP binding domain. *J. Biol. Chem.* **273**: 34631-34638. (# citations - 37) IF=5.854 (Rank 38 of 261 in the category Biochemistry and Molecular Biology). Nia Tatsis was co-mentored by Dr. Macara and I.

Poteet-Smith, C.E., Smith, J. A., **Lannigan, D.A.**, Freed, T.A. and Sturgill, T.W. (1999) Generation of constitutively active p90 ribosomal S6 kinase *in vivo*; implications for the MAP kinase-activated protein kinase family. *J. Biol. Chem.* **274**: 22135-22138. (# citations -33) IF=5.854 (Rank 38 of 261 in the category Biochemistry and Molecular Biology). My laboratory provided a novel phospho-specific antibody, which detects the RSK substrate, p140, in cell-based assays. I also proposed and helped analyze experiments and critically reviewed the manuscript.

Smith, J.A., Poteet-Smith, C.E., **Lannigan, D.A.**, Freed, T.A., Zoltiski, A.J. and Sturgill, T.S. (2000) Creation of a Stress-activated p90 Ribsomal S6 Kinase, *J.Biol. Chem.* **275**: 31588-31593. (# citations -14) IF=5.854 (Rank 38 of 261 in the category Biochemistry and Molecular Biology). I proposed and helped analyze experiments and critically reviewed the manuscript.

Clark, D.E., Poteet-Smith, C.E., Smith, J.A., and **Lannigan, D.A.** (2001) p90 (Rsk2) Allosterically Activates Estrogen Receptor α by Docking to the Hormone Binding Domain. *EMBO J.* **20**:3484-3494. (# citations- 20) IF=10.053 (Rank 15 of 261 in the category Biochemistry and Molecular Biology).

Lannigan, D.A. (2003) Estrogen Receptor Phosphorylation. *Steroids* **68**:1-9. (# citations -65), IF=2.416 (Rank 42 of 89 in the category Endocrinology and Metabolism).

Henrich, L.M., Smith, J.A., Kitt, D., Errington, T., Nguyen, B., Traish, A.M., and **Lannigan, D.A.** (2003). Extracellular Signal-Regulated Kinase 7, a Regulator of Hormone-Dependent Estrogen Receptor Destruction. *Mol. Cell. Biol.* **23**:5979-5988. (# citations -10) IF=7.093 (Rank 19 of 153 in Cell Biology Category, Rank 27 of 263 in Biochemistry and Molecular Biology Category).

Smith, J.A., Poteet-Smith, C.E., Xu, Y., Errington, T.M., Hecht, S.M. and **Lannigan, D.A.** (2005) Identification of the first specific inhibitor of p90 ribosomal S6 kinase (RSK) reveals an unexpected role for RSK in cancer cell proliferation. Cancer Res.**65**:1027-1034. (featured on the front cover side bar) (# citations -11) IF=7.616 (Rank 11 of 123 in the category Oncology).

Clark, D.E., Errington, T.E.,Smith, J.A.,Frierson, H., Weber, M.J. and **Lannigan, D.A.** (2005) The Ser/Thr kinase, RSK, is an important regulator of prostate cancer cell proliferation. Cancer Res. **65**:3108-3116. (# citations -6) IF=7.616 (Rank 11 of 123 in the category Oncology).

De, S., Macara, I.G. and **Lannigan, D.A.** (2005) Novel Biosensors for the Detection of Estrogen Receptor Ligands. J. Steroid Biochem. Mol. Biol. 96:235-244. IF=2.866 (Rank 34 of 89 in the category Endocrinology and Metabolism).

Xu, Y., Smith, J.A., **Lannigan, D.A.** and Hecht, S.M. (2006) Three acetylated flavonol glycosides from Forsteronia Refracta that specifically inhibit p90 RSK. Med. Chem. **14**:3974-3979. IF=2.286 (Rank 10 of 34 in the category Medicinal Chemistry). My laboratory developed and performed all of the bio-assays and generated all of the biological data.

Smith, J.A., Maloney, D.J., Clark, D.E., Xu, Y., Hecht, S.M. and **Lannigan, D.A.** (2006) Influence of rhamnose substituents on the potency of SL0101, an inhibitor of the Ser/Thr kinase, RSK. Bioorg. Med. Chem. 14:6034-6042. IF=2.286 (Rank 10 of 34 in the Medicinal Chemistry Category)

Nguyen, T.L., Gussio, R., Smith, J.A., **Lannigan, D.A.**, Hecht, S.M., Scudiero, D.A., Shoemaker, R.H. and Zaharevitz, D.W. (2006) Homology model of RSK2 N-terminal kinase domain, structure-based identification of novel RSK2 inhibitors, and preliminary common pharmacophore. Bioorg. Med. Chem. 14:6097-6105. IF=2.286 (Rank 10 of 34 in the category Medicinal Chemistry) My laboratory provided all of the biological data necessary to create the *in silico* model.

Smith, J.A., Maloney, D.J., Hecht, S.M., **Lannigan, D.A.** (2007) Structural basis for the activity of the RSK-specific inhibitor, SL0101. Bioorg. Med. Chem. 15:5018-5034. IF=2.286 (Rank 10 of 34 in the Medicinal Chemistry Category)

Flynn, J.M., **Lannigan, D.A.**, Clark, D.E., Cammarata, P.R. (in preparation) RNA Suppression of p42MAPK Leads to Collapse of Mitochondrial Membrane Potential with Acute Oxidative Stress in Human Lens Epithelial Cells.

Nguyen, T.L., Gussio, R., Smith, J.A., **Lannigan, D.A.**, Hecht, S.M., Scudiero, D.A., Shoemaker, R.H. and Zaharevitz, D.W. (submitted to J. Med. Chem.) Inactivation of ribosomal s6 kinase 2 (RSK2) by a chemically diverse set of established serine/threonine kinase inhibitors: mapping the binding interactions in the ATP pocket using molecular modeling.

Eisinger-Mathason, K., Andrade, J., Groehler, A.L, Muratore, T.L, Smith, J.A., Clark, D.E., Shabanowitz, J., Hunt, D.F., Macara, I.G., **Lannigan, D.A.** (submitted to Mol. Cell.) The apoptosis promoting factor, TIA-1, controls proliferation through spatial restriction of RSK2 to stress granules.

INVITED PRESENTATIONS

2007	The Endocrine Society ENDO 2007 Toronto, Ont. CA Receptor Signaling Cross Talk Symposium "The Ser/Thr Protein Kinase, RSK, and Breast Cancer"
2007	Office of Navy Research Code 30 ISR 6.1, Arlington, VA
2007	University of North Texas Health Science Center, Fort Worth, TX
2006	Boston University, MA
2006	Office of Navy Research Materials for Forensic Sensing, Arlington, VA
2006	9th Annual Army Landmine/UXO Technical Review Meeting, Springfield, VA
2005	Keystone Symposium "Hormonal Regulation of Tumorigenesis", Monterey, CA
2004	Prostate Cancer Foundation, Incline Village, NV
2004	Frederick Cancer Research and Development Center,Rockville, MD
2004	National Cancer Institute – Drug Development Group, Rockville, MD
2004	Army Research Office Workshop for the On-Chip Identification of Biological and Chemical Molecules, Raleigh, NC
2003	National Cancer Institute Breast Cancer Faculty Retreat, Warrenton,VA
2003	Cap CURE Scientific Retreat, NY, NY
2002	23rd Army Science Conference, Orlando, FL
1995, 2000, 2002	University of Virginia, VA
1998	Argonex, Charlottesville, VA
1998	Rohm & Haas, Philadelphia, PA
1998	Keystone Symposium "Nuclear Receptor Gene Family" Incline Village, NY
1999	University of Kansas, MO
1995	Exhibit presentation at the Society for Neuroscience, San Diego, CA for Receptor Technologies, Inc.
1995	Presentation to raise Venture capital at Vermont Technology Forum for Receptor Technologies, Inc.
1990,1993	University of Vermont, VT
1990	SUNY at Buffalo, NY
1990	Boston University, MA
1990	University of Edmonton, AB

CONSULTATIONS

2001, 2006, 2007	Office of Naval Research
2003-present	Luna Quest, Inc.
1999- present	Luna Innovations, Inc.
2000- 2001	Upstate Biotechnology

COMMUNITY SERVICE

1995 Television interview on CBS "Across the Fence" about my research activities.

1993-1996 New England Board of Higher Education, Mentoring Committee

1993-1996 New England Board of Higher Education, Minority Advising Committee

1999-2006 **American Cancer Society** –

May 28, 1999, I spoke at the ACS sponsored "Relay for Life" by the Shenandoah Unit of Virginia, to an audience of ~ 200 people to discuss the importance of ACS dollars to fund research.

August 19, 1999, I spoke at the Annual Meeting of the Shenandoah Unit of the ACS to discuss the importance of ACS dollars to fund research.

October 16, 1999, Lay talk about my ACS supported research to ~ 30 people as part of their training as ACS volunteers.

January 2000, the ACS placed an advertising insert in Richmond Times-Dispatch that featured my research.

September 30, 2001, I spoke at the ACS "Pink Ribbon Classic" in Richmond, VA to discuss the importance of ACS dollars to fund research.

October 27, 2001, Lay talk about my research at the Breast Cancer Forum in Fredricksburg, VA, to an audience of ~ 50 people.

Dec. 12, 2002, Lay talk about my research to ~ 30 ACS volunteers.

April 4, 2003, Lay talk to the Univ. Virginia Student's "Relay for Life" to discuss the importance of ACS dollars to fund research.

Dec. 6, 2004, Lay talk about my research at the local American Cancer Society Board Meeting.

June 11, 2005 – Lay talk about my research in support of the American Cancer Society at the Pink Ribbon Polo Cup Festivities.

April 27, 2006- Lay presentation about my research at the American Cancer Society Volunteer Recognition Program at the Univ. Virginia.

2003-2005 **Patients and Friends of the Cancer Center-**

April 3rd, 2003; July 2, 2003; Sept. 9, 2003; Feb 20, 2004; Lay talk about my research and a personal tour of my laboratory to help raise money for the Cancer Center.

October 9, 2003 – Lay talk about my research at the King Family Vineyard to help raise money for the Cancer Center.

February 29, 2004 – Lay talk about my research at Hamilton's to help raise money for the Cancer Center.

Dec. 1, 2004-Lay talk about my research at Richmond, VA in support of the Patients and Friends of the Cancer Center, to an audience of ~50 people.

April 9, 2003; Jan. 12, 2005 - Lay talk about my research to the Cancer Center Board.

June 23, 2004 – Lay talk about my research to ~ 30 people at the Health System Partner Event to help raise money for the Cancer Center.

Jan. 31, 2005- Personal tour of my laboratory in support of the Cancer Center.

Jan/Feb 2005 – Multiple interviews about my research for the newsmedia: Daily Progress, CBS WCAV 19, NBC WVIR, NPR Roanoke, Hungarian Daily Newspaper.

March 11, 2005 – Lay talk about my research as part of a film documentary on Breast Cancer Research, which is called "Summer Running".

Feb. 6, 2007 – Interview to CBS WCAV 19 about my research.

April 20,2007 – Lay talk about my research for the Annual Giving Advisory Board Dinner.